Supporting Information

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SI Materials and Methods

To validate the specificity of the PSD95 staining, immunostaining of arrays was carried out as described in *Materials and Methods*, with primary antibody combinations of PSD95 (Abcam) and NMDA receptor subunit NR2A (Millipore) or PSD95 (Abcam) and synaptophysin (Synaptic Systems), and secondary antibodies donkey anti-goat Alexa Fluor 488 and donkey anti-mouse Cy3. For confirmation of NAB antibody staining amyloid β (Aβ), we stained arrays with R1282 [provided by Dennis Selkoe (1)] and NAB61. To characterize further the NAB61 antibody, SDS/PAGE and Western blot analyses were conducted on mouse brain homogenates from APP_{Swe} transgenic (Tg2576 line; ref. 2), wild-type mice, and lysates of mouse N2a neuroblastoma cells. Immunoprecipitation of Aβ from medium of Tg2576 primary neuronal cultures also was performed by using R1282 followed by SDS/PAGE and Western blot analysis with NAB61 or 6E10 (another anti-Aβ antibody, provided by Elan), as described in refs. 3 and 4.

1. Citron M, *et al.* (1996) Evidence that the 42- and 40-amino acid forms of amyloid β protein are generated from the β-amyloid precursor protein by different protease activities. *Proc Natl Acad Sci USA* 93:13170–13175.
2. Hsiao K, *et al.* (1996) Correlative memory deficits, Aβ elevation, and amyloid plaques in transgenic mice. *Science* 274:99–102.
3. Walsh DM, *et al.* (2005) Certain inhibitors of synthetic amyloid β-peptide (Aβ) fibrillogenesis block oligomerization of natural Aβ and thereby rescue long-term potentiation. *J Neurosci* 25:2455–2462.
4. Townsend M, Shankar GM, Mehta T, Walsh DM, Selkoe DJ (2006) Effects of secreted oligomers of amyloid β-protein on hippocampal synaptic plasticity: A potent role for trimers. *J Physiol* 572:477–492.
5. Lee EB, *et al.* (2006) *J Biol Chem* 281:4292–4299.
6. Gomez-Isla T, Spires T, de Calignon A, Hyman BT (2008) in *Handbook of Clinical Neurology*, eds Aminoff MJ, Boller F, Swaab DF (Elsevier, Edinburgh), Vol 89, pp 233–243.

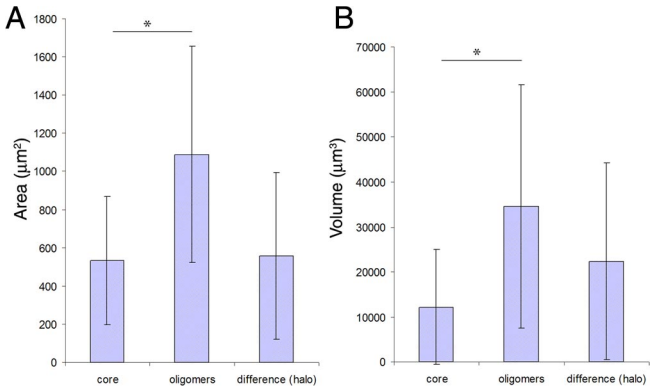


Fig. S1. A halo of oligomeric Aβ surrounds dense plaques. (*A*) Quantification of the area of methoxy XO4 and NAB61 staining postmortem shows that oligomeric Aβ occupies double the area of the core alone, with a halo extending ≈5% beyond the area of the core. (*B*) Projected volumes assuming spherical shape show that the halo of oligomeric Aβ occupies almost twice the volume of the dense core (*, $P < 0.0001$, Student's *t* test).

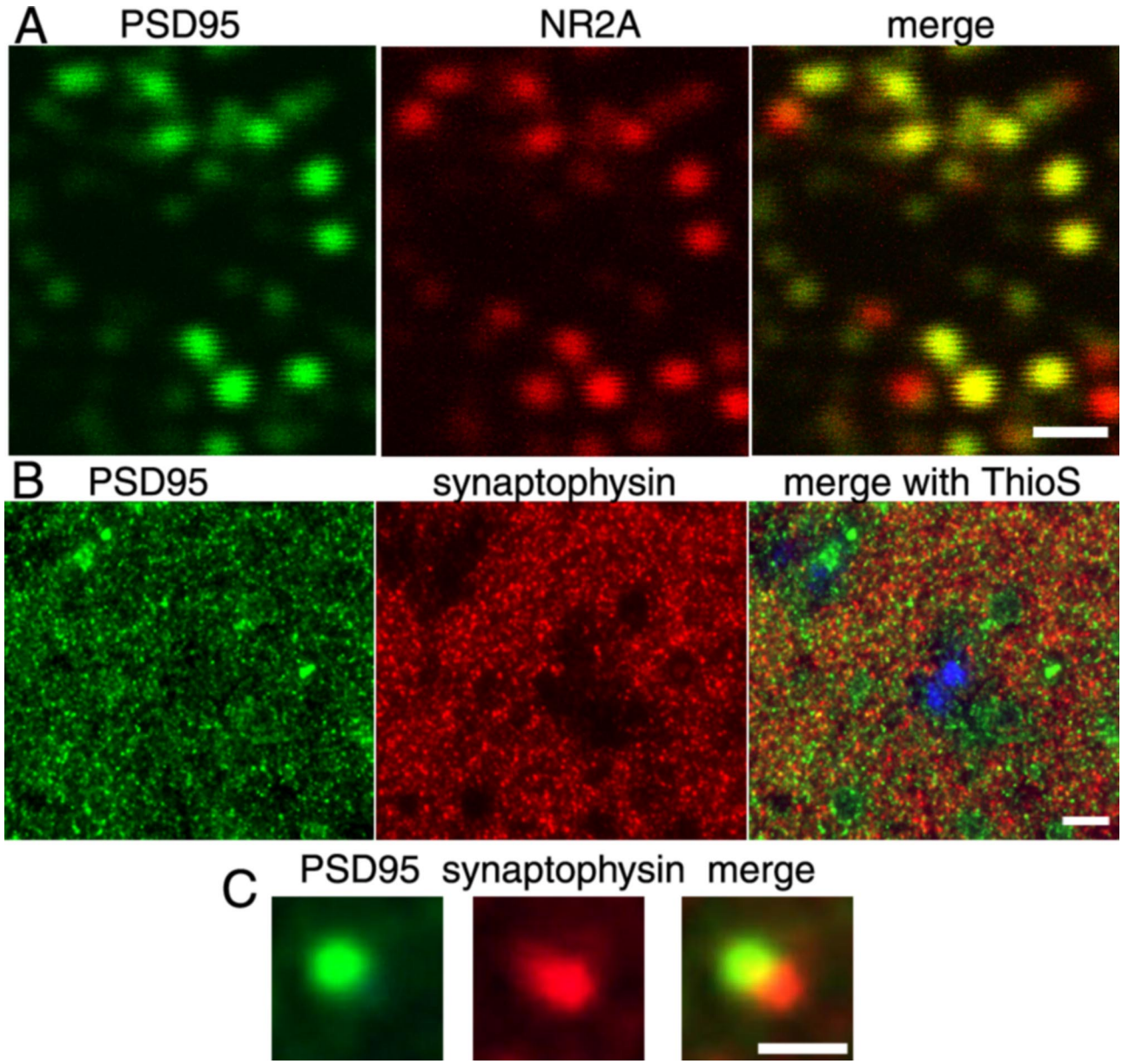


Fig. S2. Confirmation of PSD95 as a reliable excitatory postsynaptic marker. (*A*) PSD95 was costained with the NR2A subunit of the NMDA receptor, which is known to bind the PDZ domain of PSD95. These two proteins showed excellent colocalization. (*B*) Costaining with presynaptic marker synaptophysin shows a similar loss of presynaptic material in plaque cores (ThioS; blue) as described for PSD95. (*C*) It also confirms that excitatory PSDs are opposed by a presynaptic terminal. Together, these data strongly suggest that the PSD95 antibody used is a reliable marker of excitatory PSDs. (Scale bars: *A* and *C*, 1 μm; *B*, 10 μm.)

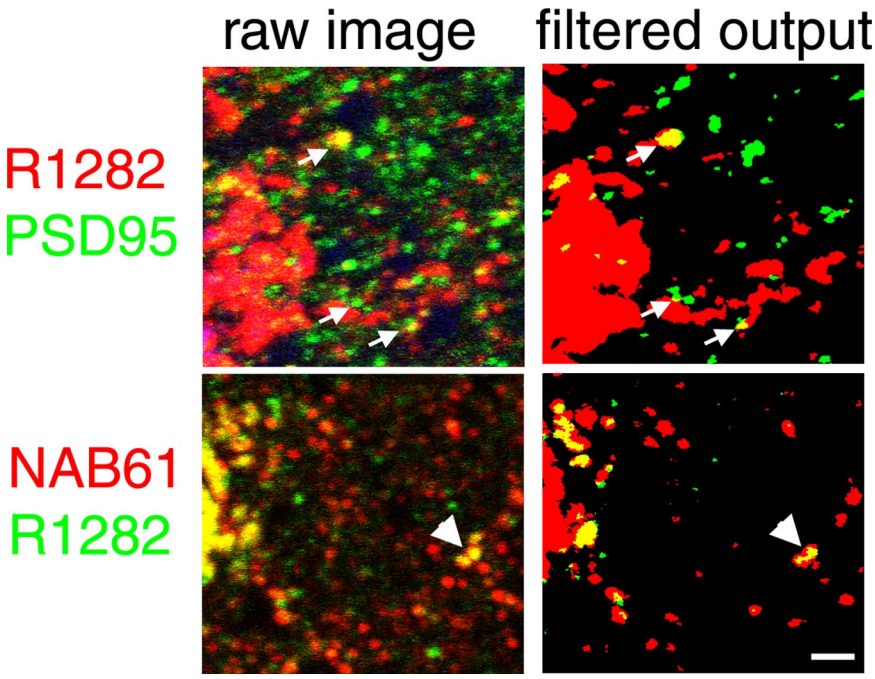


Fig. S3. Confirmation that another marker that recognizes oligomeric Aβ costains with PSD95 and NAB61. Double-immunofluorescence labeling of R1282, an antibody that has been shown previously to react with oligomeric species (3, 4), with PSD95 shows that this antibody also labels the neuropil in a punctate manner and that these puncta contact a subset of PSD95-labeled synapses (*Upper*, arrows). Costaining with NAB61 (*Lower*) shows that although R1282 does not label as many puncta as NAB61, most of those that are R1282-positive costain with NAB61 (arrowheads). (Scale bar: 2 μm.)

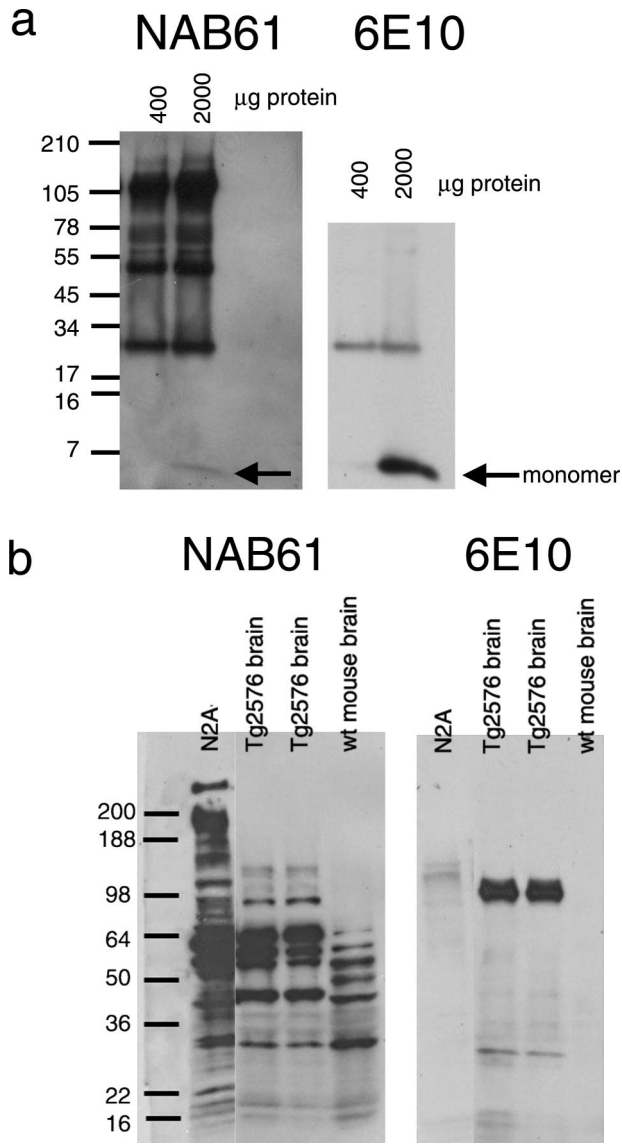


Fig. S4. Biochemical characterization of the NAB61 antibody. In the original work describing the NAB61 antibody, it was shown to preferentially bind oligomeric over monomeric species of synthetic Aβ (5). (*a*) To confirm this, Aβ was immunoprecipitated from media of Tg2576 primary neuronal cultures with R1282 antibody and then subjected to SDS/PAGE and Western blot analysis with both NAB61 and 6E10. The 6E10 primarily detected Aβ monomers (arrows), whereas NAB61 preferentially recognized larger, presumably oligomeric, species of Aβ. (*b*) SDS/PAGE and Western blotting show that NAB61 recognizes SDS-insoluble Aβ oligomers from mouse N2A cell line lysates and in both Tg2576 brain homogenate and wild-type brain homogenate. Both the N2A and mouse brain data indicate that NAB61 recognizes endogenous mouse Aβ. 6E10 N-terminal antibody that recognizes human Aβ was run on an identical gel, showing that it does not recognize mouse Aβ.

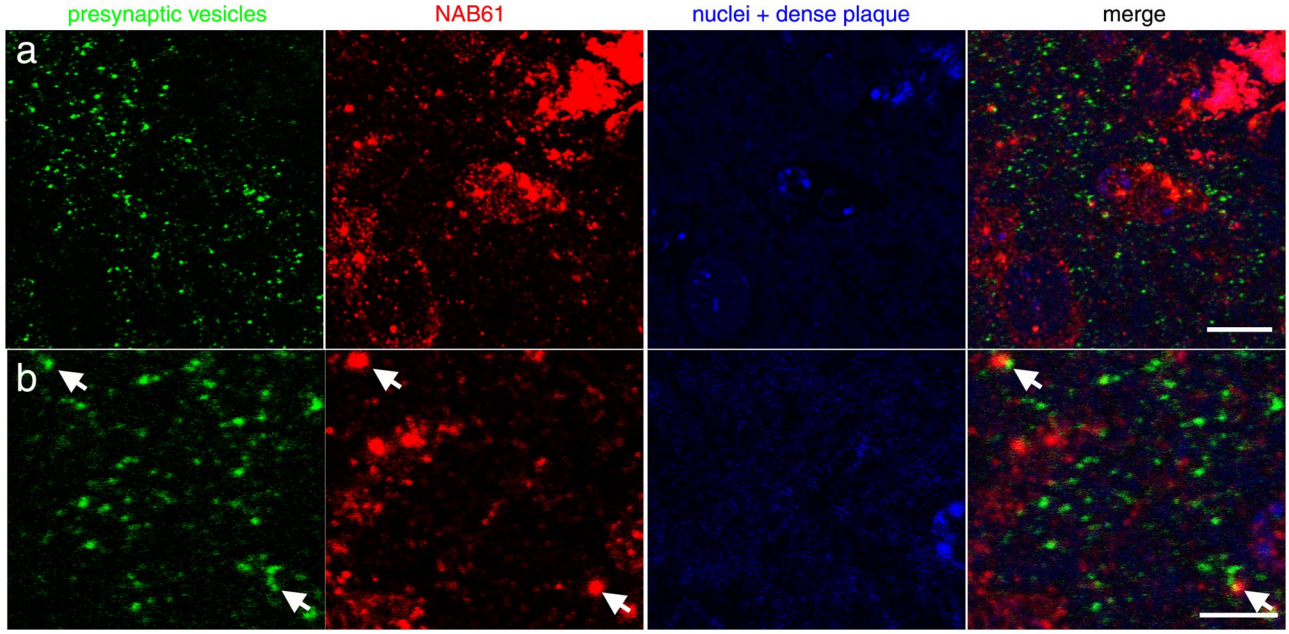
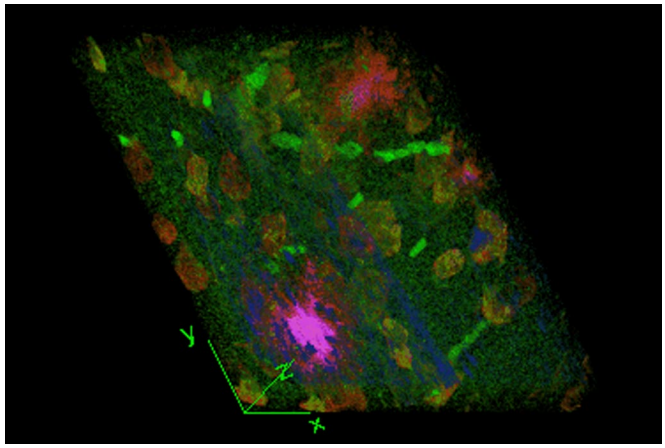


Fig. S5. Colocalization of a presynaptic marker with NAB61 puncta. Loss of presynaptic material has been extensively reported in AD associated with plaques (6). We confirm by using synapsin I staining that presynaptic markers are drastically reduced in the dense cores of plaques (*a*). At higher resolution (*b*), we also observed colocalization of NAB61 puncta with synapsin I-labeled synapses (arrows). (Scale bars: *Upper*, 10 μm; *Lower*, 5 μm.)



Movie S1. Rotation of a 3D reconstruction (Image J) of staining with NAB61 (red), PSD95 (green), and ThioS (blue) demonstrates the localization of oligomeric Aβ to plaque cores, a halo around plaques, cell bodies, and small puncta in the neuropil.

Movie S1 (GIF)